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Electrochemical sensor for analysis of analytes in liquids

This invention relates generally to the analysis of analytes in fluid samples, and in a particular aspect provides an electrochemical sensor for use in such analyses.

It is known to assay biological fluid samples such as whole blood, plasma, or serum, for a plurality of different analytes therein. Generally, such testing has been carried out by continuous-flow systems such as those shown in the U.S. Patent NOs. US—A—2,797,149, 3,241,432, 3,479,141 and 3,518,009. Also, chemical testing of ionic analytes has been performed in an automated fashion using thin films of material, such as is shown in U.S. Patent No. US—A—4,053,381.

In order to perform blood testing, a great number and variety of tests have to be made. This requires many electrochemical cells of different structures and chemistries. Rapid and cost-effective methods of analysis require a simultaneous analysis of all the analytes in a fluid sample. Emphasis must also be directed to reduction of the sample size, preferably to a few drops or less of blood to minimise demands on the subject, e.g. in the case of infants.

A device that suggests an integrated circuit approach for the testing of a variety of blood analytes in a sample is shown in U.S. Patent No. US—A—4,020,830. This device features an integrated array of field effect transistors (FETs), each designed as a discrete sensor. While this is a valid approach to the automated testing of blood samples, certain shortcomings are inherent in this technique:

(a) Only ion-selective FETs have been successfully and reliably demonstrated. When designed to measure non-ionic analytes, the FET structure becomes very complex, because an additional electrochemical cell must be placed at the gate electrode of the FET to influence the measured drain current. This measurement, however, requires a constant current source in addition to the cell FET and external reference electrode.

(b) Instability in any complement will naturally cause fluctuations in the drain current, and, hence, errors in the measurement of the analyte. In addition, the proposed enzyme and immuno FETs have polymer layers, where concurrent processes such as adsorption and ionic double layer capacitance changes can affect the electric field at the gate of the FETs. Extraneous electric fields are also produced at the fringes of the gate area. These effects will likewise cause errors in the analysis of the analytes.

(c) The need for an external reference electrode when measuring non-ionic analytes complicates the integration of a FET array.

(d) FETs will only detect a charged molecule, i.e. an ion. Non-charged analytes do not influence the gate voltage in an interference-free manner. Hence, analytes which can be successfully analysed are limited.

However, the semiconductor fabrication technology is so advanced that very precise miniature devices can be easily and cheaply manufactured. Furthermore, precedence has been established for superior stability, reproducibility and sensitivity. Hence, in various aspects of the present invention, we seek to combine the best attributes of two technologies (electrochemistry and semiconductors) to achieve integration of sensors without the drawbacks and limitations of the FET approach.

One particular problem associated with the use of FETs is that in paragraph (c) above, namely the necessity for an external reference electrode. According to the present invention, we have devised an electrochemical sensor which does not require an external reference electrode since it has a built-in calibration device.

In U.S. patent No. US—A—4053381, certain electrochemical sensors in the form of ion-selective electrodes are described which consist of a pair of identical electrodes mounted in spaced relationships, each electrode comprising a layer of permeable material which contains the same species as is to be measured, and which (in each electrode) is accessible to an externally applied liquid. In use, a drop of the liquid sample to be analysed is placed in contact with one electrode, and a drop of standard reference solution is placed in contact with the other (identical) electrode, and from signals produced a measure of the species to be assayed in the liquid sample is obtained.

We have now devised an electrochemical sensor which does not require the use of an externally applied reference liquid.

The present invention provides an electrochemical sensor for use in the analysis of an analyte in a liquid sample, the sensor comprising at least a first and second electrode and means for mounting the said electrodes in spaced relationship, the first electrode being electrically connected with a first layer of permeable material which contains a given concentration of the species to be measured, and the second electrode being electrically connected with a second layer of permeable material which is accessible to the species to be measured as derived from the liquid sample, characterised in that the second layer is either free from the said species or contains a concentration thereof different from that in the first layer.

The invention also provides apparatus for simultaneously analysing a multiplicity of analytes in a fluid sample, the apparatus comprising an array of discrete, electrically isolated electrochemical sensors for analysing different analytes; all the sensors being

supported on a common substrate, and electrical circuitry for obtaining the signal(s) from each sensor, characterised in that at least one of the sensors is as defined above.

The invention further provides apparatus for use in analysing a multiplicity of analytes in a fluid sample, comprising a plurality of discrete, electrically isolated sensors of the invention adapted to receive reaction products, said sensors being supported on a common substrate, each of said sensors being specific to a particular reaction product, characterised by a reaction cell for selectively reacting particular constituents in a liquid sample to form said reaction products with each of said constituents.

The array of discrete, electrically isolated sensors may include, in addition to sensors of the invention, sensors of other different constructions.

Electrochemical sensors of the invention, optionally together with other electrochemical sensors not according to the invention can conveniently be assembled as a micro-miniaturised, multi-functional, electro-chemical, integrated circuit chip for analysing concurrently a plurality of analytes in a minimal sample volume. The circuit chip comprises a substrate supporting a plurality of individual sensors arranged in a dense but discrete relationship to form an integrated array. Unlike integrated sensors arrays of the prior art, which provide a single common reference electrode, the present invention appreciates that a more reliable analysis results when an electro-chemical sensor has its own reference electrode. Normally, it would be expected that the use of separate reference electrodes for each sensor is an unnecessary duplication of components. The present invention, however, achieves this result while providing a more compact chip, which is of relatively simple fabrication.

The circuit chips may be a combination of any one or more of three types of electro-chemical cells: (a) a current measuring cell; (b) a potential measuring cell; or (c) a kinetic rate measuring cell. Some of the electro-chemical sensors will be ion-selective and adapted to measure ions, such as Na+ or K+, potentiometrically. Other sensors may be adapted to measure a redox reaction for the detection of glucose, LDH, etc., by amperometric/voltammetric methods.

A small, hand-held computer is used to analyse, or "read out", and display the measurements of each of a plurality of analytes in the fluid sample.

Each electro-chemical sensor is selective with respect to only one analyte. For example, such selectivity may be achieved by providing each sensor with a first porous medium or gel layer containing an immobilised enzyme, specific for only one analyte in the sample. The first porous layer is combined, in some cases, with a second porous filtering layer to selectively screen the fluid sample for a particular analyte. In other cases, the first porous layer functions as a filter to extract the desired analyte from the fluid sample. The first porous layer may also contain a substance to extract the particular analyte and/or make the analyte more soluble in the porous medium, such that the analyte will prefer the porous medium to that of the fluid sample.

A barrier or encapsulating layer may be provided for the circuit chip to preserve its shelf-life and to protect against environmental or external contamination. In one embodiment, the encapsulating layer can comprise a tear-away, impermeable envelope or mantle. In another embodiment, the barrier layer can comprise a semipermeable filter layer for preventing contamination and for removing high molecular weight molecules or other particles that may interfere with the chemical analyses of the fluid sample, e.g. red cells in whole blood.

The integrated chip can be typically fabricated, as follows:

(a) a substrate is formed by press-forming a material such as powdered alumina with appropriate thru-holes and imprints for the electro-chemical circuit; the pressed alumina powder is then fired;

(b) the thru-holes are then filled with conductive material, e.g. pyrolytic carbon;

(c) on the back-side of the substrate, a wiring pattern is deposited using conventional photo-resist etching techniques;

(d) on the front-side of the substrate, a pattern of sensor wells is formed by conventional photoresist etching techniques;

(e) with a series of masks, the appropriate layers for each sensor are built up. These layers may comprise polymers or gels including appropriate reagents, i.e. enzymes, and other appropriate substances;

(f) the entire chip is then protected by a coating of epoxy or thermoplastic, with the exception of the sample contact area of the sensors;

(g) a protective barrier is then placed over the sensors.

Generally speaking the sensor of this invention features the following advantages over the prior art:

(a) The circuit chip is intended as a disposable device, and, therefore, does not suffer from "prior sample memory" problems associated with prior art electro-chemical sensors.

(b) The electro-chemical sensors of the invention include a self-contained calibrating solution to stabilise their particular chemical activity. The calibrating solution contains a known quantity of analyte (or species to be measured) and may be impregnated in one of the porous layers of the electro-chemical sensor, which is adapted to minimise capacitive and impedance effects, and eliminates the need of calibrating each test in every sample.

For example, in the measurement of potassium, two identical potassium sensing electrodes are incorporated in a single sensor structure and used in a differential mode in such a way that external reference electrodes are not required. The layer of the sensor contacting the sample and associated with the sample sensing electrode contains a low concentration of potassium ion (e.g. 1.0 mEq/L.). The layer associated with the other electrode, which is not in contact with the sample, contains a high concentration of potassium ion (e.g., 5.0 mEq/L). The difference in potassium ion concentration allows calibration of the sensor for sensitivity prior to sample introduction while the differential EMF measurement procedure minimises signal drift during sample measurement.

In a sensor for the measurement of BUN (blood urea nitrogen), as another example, appropriate layers are similarly impregnated with high and low concentrations of the species NH_4+. Additional NH_4+ generated by the urease-gel layer results in a change in the differential signal. The self-calibrating sensors also provide ease of fabrication of the circuit clip by reducing the manufacturing tolerances required for the gel layers and electrode structures, because electrodes realistically can never be perfectly matched.

(c) The self-contained integrated structure of electro-chemical sensors disposed and interconnected on a common substrate eliminates effects common to other multiple-sensor arrangements, such as liquid junction effects, electrolyte streaming effects and electro-kinetic phenomen. In addition such structure is more compact and easily fabricated.

(d) The barrier layer or encapsulation ensures that the circuit chip can have an extended shelf-life by preventing environmental and external contamination.

(e) Signal-to-noise characteristics are improved, as noise sources can be eliminated.

(f) Chemical noise is minimised by confining substances to polymer or gel layers.

(g) Thermal and mass transport gradients are minimised by the commonality of substrates, construction materials, and the miniaturisation of the sensing elements.

(h) Each circuit chip may be made to interface with a small, hand-held computer, by means of snap-in connections thus providing on site analysing convenience and portability.

(i) As stated previously a circuit chip of the invention may include, in addition to electro-chemical sensors of the invention, one or more electrochemical sensors. Of these, sensors for measuring enzyme analytes may feature a new method of analysis and a new sensor construction based upon this new analysing technique, which comprises;

(1) electrically generating a reactant of said enzyme reaction to establish a steady state condition for the reaction; and

(2) electrically monitoring the enzyme reaction to control the generation of the reactant and establish the steady state condition.

The new sensor construction capable of performing this new technique includes: a generating electrode a monitoring electrode and a reaction medium disposed therebetween. The steady state is achieved as a result of the rate of reagent formation and rate of depletion by the enzyme reaction.

In order that the invention may be more fully understood, various embodiments thereof will now be described, by way of example only, with reference to the accompanying drawings, in which:

FIGURE 1 is a perspective view of an integrate substrate supported chip of this invention shown with an encapsulating layer being removed therefrom;

FIGURE 1*a* is a side view of an alternate encapsulating embodiment to that depicted in Figure1;

FIGURE 2 is a cut-away perspective view of the substrate supported chip of Figure 1 being deposited with a drop of blood;

FIGURE 3 is a perspective view of a hand-held analyser or computer for receipt of the substrate supported chip of Figure 2, and for analysis of the fluid deposited upon the chip;

FIGURE 3*a* is a side view of Figure 3;

FIGURE 4 is a schematic, enlarged plan view of the sensor array on the typical chip of Figure 1;

FIGURE 5 is a further enlarged cross-sectional view taken along lines 5—5 of a typical row of sensors of the array of sensors shown in Figure 4;

FIGURE 6 is an enlarged partial schematic wiring diagram for the typical row of sensors depicted in Figure 5;

FIGURES 7*a* through 7*d* are further enlarged cross-sectional views of the typical sensors illustrated in Figure 5;

FIGURE 7*a* shows a typical current measuring cell (not a cell of the invention) with immobilised enzyme in a gel layer for potassium ion measurement;

FIGURE 7*b* depicts a typical kinetic measuring cell (not a cell of the invention) for LDH measurement;

FIGURE 7*c* illustrates a typical ion-selective cell of the invention with immobilised enzyme in a gel layer for potassium ion measurement;

FIGURE 7*d* shows a typical potential measuring cell of the invention for BUN measurement;

FIGURE 8 is an enlarged cut-away perspective view of a typical sensor assembly of Figure 4;

FIGURE 8*a* is a perspective partial view of the electrode-substrate-circuit construction of Figure 8;

FIGURE 9 is a schematic electrical diagram of a conditioning circuit for the output of the

enzyme sensor shown in Figure 7b;

FIGURE 10 is a schematic diagram for the analyser depicted in Figures 3 and 3*a*;

FIGURE 10*a* is a more detailed schematic diagram for a portion of the circuit of Figure 10;

FIGURE 11 is a schematic diagram of a continuous flow system for analysing a fluid using a modified chip as that illustrated in Figure 4;

FIGURE 12 is a schematic diagram of a continuous system of using thin films to form a plurality of fluid analysing sensors including sensors of the invention;

FIGURE 13 is an enlarged cross-sectional view of the films depicted in Figure 12; and

FIGURE 14 is an enlarged plan view of the film shown in Figure 13.

While the invention is primarily directed and described with reference to blood analyses, it should be understood that a great variety of fluid samples can be analysed by modifying the sensor chemistries.

Referring to Figures 1 and 1*a*, a circuit chip 10 for analysing a fluid sample is shown in an enlarged view. The chip 10 is disposed within a hand-held tray support 11. The chip 10 and tray support 11 are both covered by an encapsulating barrier 12 that can either be in the form of a peel-off layer 12*a* of Figure 1, or a separable encapsulation envelope 12*b* of Figure 1*a*. The barrier 12 may also take the form of a built-in semi-impermeable layer of membrane 12*c* of Figures 1*a* and 2. The semi-impermeable membrane 12*c* may also act as a filter, for removing high molecular weight molecules or particles, such as red blood cells. The barrier, regardless of structure, excludes contaminants from chip 10, and thus preserves its reliability and shelf-life. The circuit chip 10 is composed of an array or plurality of spaced-apart sensors 14 which consist of, or include, sensors of the invention. The sensors may be planar shaped or designed as miniature cups or wells to receive a drop of blood 13 deposited on the chip 10, as illustrated in Figure 2. Each sensor 14 is designed and constructed to be specific to a particular analyte in the fluid blood sample 13. This is generally achieved by including within each sensor 14, an enzyme or catalyst that initiates a characteristic reaction. The particular chemistries, reagents, materials, and constructions for each sensor 14 are described in more detail hereinafter.

The hand-held support 11 for the chip 10 comprises a flat base surface 15 and vertically tapered side walls 16 extending from surface 15 for supporting the chip 10 and directing fluid sample 13 into wetting contact with chip 10 and sensors 14. The side walls 16 may be coated with hydrophobic material and serve as a sample confining structure. These side walls 16 define a perimeter of the chip circuit and the outer boundaries of liquid/chip contact.

Obviously, other designs are achievable within the objectives set forth above, such as, for example, a circular retaining well to replace the square-shaped well defined by walls 16, or a planar boundary wall flush with the surface of the chip (not shown).

The tray support 11 and chip 10 are designed to hold a small volume of sample fluid, i.e., one drop or less. Thus, a finger 17 can be placed directly over the chip 10 and pricked so as to dispense a drop of blood 13 directly onto the chip, as illustrated in Figure 2. The blood drop 13 spreads over the entire chip 10, to simultaneously wet all sensor sites 14. Because chip 10 is miniaturised, a minimal amount of blood sample will coat the entire sensor surface 18.

Each electro-chemical sensor 14 has a different number of electrodes 22 (Figures 5, 8 and 8*a*) depending upon whether its chemical reaction is measureable as a kinetic rate, a current change or a potential change. The electrodes 22 of each sensor 14 are deposited upon a common substrate 20 of the chip 10, as shown in Figures 7*a*—7*d*, 8 and 8*a*, so as to provide a compact and easily fabricated structure. An interconnection circuit 24 is deposited on the opposite side of the common substrate 20 to which all the electrodes 22 are electrically connected as illustrated in Figures 8 and 8*a*. The use of two surfaces of a common substrate 20 for all the electrodes 22 of each sensor 14 and the signal receiving wires 25 of circuit 24 (Figure 8*a*) provide a self-contained, integrated array of sensors 14 unique to chip constructions of this type.

Figure 4 shows a greatly enlarged schematic plan view of a chip 10 having a typical sensor array. Sixteen sensor sites 14 are depicted, by way of illustration. Each sensor 14 may be symmetrically spaced-apart from the other sensors 14, but this symmetry is not of a functional necessity. Each sensor 14 has a group of electrical interconnectors 25 (Figures 4 and 8*a*) forming part of the interconnection circuit 24. The number of interconnections 25*a*, 25*b*, 25*c*, 25*d*, etc. for each sensor 14 in a typical sensor row, as shown in Figure 6, depends upon the type of sensor 14*a*, 14*b*, 14*c* and 14*d*, (Figures 5 and 6), respectively, being interconnected, as will be described in more detail hereinafter.

The interconnectors 25 each terminate in an electrical connection 27 projecting from the end 26 of chip 10 (Figures 1, 3 and 4), which is adapted to mate with a snap-in electrical connector 28 disposed in slot 29 of an analysing device 30. The connection 27 of chip 10 overhangs the tray 11, as illustrated, and includes a slot 31 for keying into connector 28 of analyser 30.

The analysing device 30 (Figures 3 and 3*a*) receives the electrical inputs from each sensor 14 on chip 10 via the snap-in connector 28. Analysing device 30 may be a hand-held computer, with a keyboard 32 and a display 33. A print-out 34 may also be provided, as shown. Certain keys 35 of keyboard 32, when

depressed, interrogate a particular sensor 14 of chip 10. Other keys 35 are adapted to initiate a programmed sequence, such as a test grouping, system calibration, sensor calibration, etc. The analysis of the blood sample 13 for a particular analyte is initiated by depression of a selected key 35 and the result is displayed in display window 33. The signal processing by the analysis device 30 is explained hereinafter with reference to Figures 9, 10 and 10*a*.

Referring to Figure 8, a perspective cutaway view of a typical sensor site is shown. First, substrate 20 is press-formed from powdered alumina. The appropriate thru-holes 48 for each sensor site 14 are defined in substrate 20. Horizontal surfaces 41 and 45 define a typical electrode area. On the bottom surface 45 of substrate 20, the interconnection circuit 24 is deposited by conventional photoresist etching techniques. Holes 48 are filled with electrode conductor material, such as pyrolytic carbon, to provide electrical connection between surfaces 41 and 45 of substrate 20. The deposition of the pyrolytic carbon is conventionally effected by an appropriate masking technique.

Interconnection circuit 24, containing conductors 25 for connecting electrodes 22 in each sensor site 14, is formed over surface 45 of substrate 20. A thin coat 46 of epoxy is layed over surface 45 to protect the interconnection circuit 24.

On the upper surface 41, a layer 50 of thermoplastic material is then deposited to form the necessary well-shaped sensor sites 14, as defined by surfaces 16, 40, 42 and 43. In some cases, (Figure 7*b*) sensor construction requires photoresist layers 44 prior to the thermoplastic well formation.

Next, the chemical layers are formed at each sensor site 14 by depositing layers 51, 52, 53, 54, etc. After layers 51, 52, 53, 54, etc. have been deposited, the chip 10, with the exception of the contact area 18 defined by borders 60 (Figures 1*a* and 2), is coated with an epoxy or thermoplastic layer 12*b* defining a support tray 11. A protective semi-permeable barrier layer 12*c* is then deposited over the blood contact area 18. If desired, the entire chip 10 and tray 11 may be overlayed with the aforementioned tear-away impermeable layer 12*a* of Figure 1, or the encapsulation envelope 12*b* of Figure 1*a*.

Now referring to Figures 5, 6 and 7*a* through 7*d*, a typical row of sensors 14*a*, 14*b*, 14*c* and 14*d* are respectively illustrated to describe four different basic sensor electro-chemistries. Each of the sensors 14*a*, 14*b*, 14*c* and 14*d* have electro-chemistries which will apply to the other similar sensors upon chip 10 and with respect to other contemplated analytes being assayed.

The sensor 14*a* is not a sensor of the invention. Its construction provides for measuring glucose (GLU) in the blood sample. The glucose in the blood will permeate and filter through the barrier layer 12*c* and a further cellulose filtering layer 70, respectively, and then diffuse into a polymer or gel layer 71*a* containing the enzyme glucose oxidase. Hydrogen peroxide is produced in layer 71*a* from the enzyme-catalysed oxidation of glucose within the polymer layer. The hydrogen peroxide diffuses through layer 71*a* to the surface 22*a* of electrode 72*a*. The concentration of hydrogen peroxide is monitored by measuring the anodic current produced at electrode 72*a* by the electrooxidation of hydrogen peroxide at +0.7 volts vs. silver/silver chloride reference electrode as applied at electrodes 72*b* vs. 72*c* and 72*a* vs. 72*c*. Alternatively, the total anodic charge may be measured. Layer 71*b* is similar to layer 71*a*, but does not contain the enzyme glucose oxidase. Therefore, as glucose diffuses through layers 12*c* and 70 into layer 71*b*, no reaction will be monitored at electrode surface 22*b* of electrode 72*b*. This electrode 72*b* acts as an error correcting electrode. The signal from electrode surface 22*b* will be subtracted from the signal of electrode surface 22*a* by differential measurement to eliminate other oxidisable interferences in the blood sample.

The reference electrode 72*c* extends in an annular fashion (shown only in cross-section here) about electrodes 72*a* and 72*b*. Thus, the surface 22*c* of electrode 72*c* is made much larger in area than electrode surfaces 22*a* and 22*b*, in order to maintain voltage stability during measurement (during current flow). Electrode 72*c* supports the current flow of sensor 14*a*. The formal potential of the electrode 72*c* is maintained by annular layer 71*c* (also only shown here in cross-section), which comprises a Cl^- containing polymer or gel (Ag/AgCl with Cl^-). The reference electrode 72*c* is the Ag/AgCl electrode couple. The respective electrodes 72*a* and 72*b* are composed of carbon and are connected electrically to respective wires 25. The annular reference electrode 72*c* may contain carbon or Ag.

Sensor 14*b* of Figure 7*b* is not according to the invention. It is designed to measure LDH in the blood sample. The chemistries used for determining LDH, as well as other enzyme analytes in blood requires that a kinetic rate be measured. In the past, kinetic rate measurements of this type always have required the measurement of time dependent parameters. Therefore, two or more readings in time or a continuous monitoring was required to obtain the kinetic rate measurement. Sensor 14*b*, however, is constructed in a new way in order to make use of a new method of measuring kinetic rate. The new method will provide a virtually immediate enzyme activity reading. Only one reading is required, and the electro-chemical sensor is not subject to electrode surface effects that will alter the calibration, nor to prior experienced changes in the electro-chemical nature of the gel composition resulting from the current flow during the measurement. Furthermore, the enzyme reaction does not occur until actuated

by a new type of current generating electrode of the sensor, as will be explained hereinafter. The sensor 14*b* is a more accurate, reliable, and convenient device for determining enzyme analytes requiring a kinetic rate measurement.

The new method features controlling the concentration of the reactants in the following LDH related enzyme reaction for a given time interval:

$$NAD+ + Lactate \xrightleftharpoons{LDH} NADH + Pyruvate + H^+$$

When the reactants are controlled, a steady state condition will apply for this extended period of time. During this steady state condition, a single measurement of the kinetic rate of the enzyme reaction will determine the activity of the LDH enzyme. Obviously, only a single measurement need be made because there will be no change in kinetic rate with time (steady state). The formation of the NAD+ is kept at a very high level to maintain maximum rate and linearity of response. A pyruvate trap is provided to force the reaction to the right and prevent a back reaction from influencing the monitored forward reaction. This is accomplished by impregnating the enzyme reaction layer with a semi-carbazide, which will react with the pyruvate product.

The LDH of the blood sample initially permeates the barrier layer 12*c* and is then diffused through a second barrier layer 80 of an electrically conductive material such as sintered titanium oxide, tin oxide or porous graphite. This barrier layer 80 also serves as the counter or auxiliary electrode of the sensor, and is connected to a wire 25 of circuit 24 by means of a current conductor 48, as aforementioned. The LDH next permeates to a gel layer 81 containing the enzyme substrate (such as lactic acid) and a coenzyme NADH. The NADH in this layer is electro-chemically converted to NAD+ by means of a generating electrode 82, which is carbon deposited within gel layer 81, as shown. Layer 81 also contains a semicarbazide for trapping the pyruvate product of the reaction. The electrode 82 receives predetermined constant current from the analysing device 30 via a wire 25 and vertical current conductor 48. The rate of formation of NAD+ will be controlled due to the predetermined constant current being fed to the generating electrode 82.

This generating rate is measurable by the monitoring electrode 84, which is positioned below the reactant generating electrode 82. However, as the LDH of the sample diffuses through layer 81 into polymer layer 83, the NAD+ which is being generated at electrode 82 will be consumed by the enzyme catalyzed reaction with the lactate substrate. The electrode 84 will now sense the rate at which the NAD+ is being reconverted to NADH. Therefore, the monitoring electrode 84 will sense the altered NAD+ generating rate. The altered current flow from that of the initial NAD+ generating rate is directly proportional to the activity of LDH in the sample. Polymer layer 83 also acts as a medium for the reference electrode of the sensor 14*b*. All the electrodes 80, 82, 83, and 84, respectively, are electrically connected to respective wires 25 via carbon conductors 48. The monitoring electrode 84 will provide the analyser 30 with an almost immediate current or charge that will be a single measurement or reading of the kinetic rate of the reaction. Reference electrode 85 comprises a film of carbon covered by a polymer layer 85*a* which contains quinone/hydroquinone to define a stable redox potential.

If the LDH or other enzyme analyte were measured the old way by taking several readings with respect to time, sensor 14*b* would be constructed more like sensor 14*a*. The new method of measurement, as applied to thin film integration, however, does not require a difficult structure to fabricate. Yet, it provides an enormous advantage of obtaining a reading in only a few seconds required for steady state conditions to be achieved. This method and sensor construction make the integrated circuit approach to blood analysis more viable than any device previously contemplated since many enzymes in the blood can be easily and quickly analysed by this approach. This is so, because this method greatly simplifies the electronics needed to determine the kinetic rate (not time base required), and it is more accurate and reliable due to the shortened period of response required to accomplish this measurement. Also, because the reagent is generated at will, the device has improved shelf-life and overall stability, i.e., the reaction starts only when the system is ready to accept data. As a result, it does not matter whether a portion of the NADH in layer 81 degrades during storage because the generation is controlled.

Sensor 14*c* illustrates a sensor construction of the invention for determining the K+ analyte in blood. After the K+ filters through the initial barrier layer 12*c*, it diffuses into a layer 90 of cellulose which is a permeable secondary and optional barrier/filter medium. The sensor 14*c* is structured as a twin electrode sensor comprised of two identical potassium sensing electrodes. The right-hand electrode 95*a* functions as a reference electrode because its potassium concentration is fixed by the gel layer 91*a* and, hence, provides a fixed half-cell potential for the lefthand electrode 95*b*.

Layer 91*a* together with layer 91*b* provides the means for sensitivity calibration of sensor 14*c*. Layers 91*a* and 91*b* each have a predetermined concentration of K+, but one which sets up a differential voltage signal between the two electrodes, e.g., layer 91*a* could have 5.0 mEq/L of K+, whereas layer 91*b* could only have 1.0 mEq/L of K+ and ideally the resulting voltage between them should be 42 mV, but for

practical purposes the voltage will vary depending primarily on fabrication irregularities. Hence, the twin electrodes 95*a* and 95*b* provide a differential measurement which allows actual sensitivity calibration prior to sample measurement and at the same time will nullify any drift and offsets in the measurement.

The cellulose layer 90 filters the blood sample to allow only K+ ion to filter to the lower layers.

Layers 12*c* and 90 are designed to allow diffusion of ions in the sample primarily into layer 91*b* where the change in voltage of electrode 95*b* yields the additional potassium introduced by the sample. Alternatively, the differences in concentrations in layers 91*a* and 91*b*, can be made so large that diffusion of sample potassium into layer 91*b* will not constitute a significant error. For example, if layer 91*a* contains 0.1 mEq./L of K+ and layer 91*b* contains 100 mEq./L of K+ then a 5 mEq./L sample would result in voltage changes of 102 mV and 1.3 mV, respectively. If uncompensated, the 1.3 mV voltage change of electrode 95*b* would only constitute an assay error of 0.2 mEq./L. However, regardless of the concentrations of K+ in layers 91*a* and 91*b*, an algorithm can be written to take into account the signal changes, however minute, in both electrodes 95*a* and 95*b*. From a practical standpoint, however, the reference side of the sensor should not change significantly in voltage relative to the other sample sensing side.

Layer 93*a* directly above the reference electrode 95*a* contains ferro/ferric-cyanide to form a stable redox couple for electrode 95*a* and also a fixed K+ concentration to maintain a stable interfacial potential between layers 93*a* and 92*a*. Layer 92*a* above layer 93*a* is polyvinyl chloride impregnated with a neutral ion carrier valinomycin, which is selective to potassium.

Layers 92*b* and 93*b*, respectively, are identical layers to their counterpart layers, 92*a* and 93*a*, with the exception of the reagents contained therein.

The calibrating layers 91*a* and 91*b*, respectively, may be maintained at a given or predetermined distance above the electrodes. Also, their thickness or size may be carefully controlled in manufacture. This will insure predetermined electrical characteristics such as capacitance and impedance for the sensor.

Sensor 14*d* depicts a sensor of the invention for the assay of Blood Urea Nitrogen (BUN).

The urea assay is accomplished by the sensing of the ammonium ion NH_4+. The urea in the blood permeates the barrier layer 12*c* and the cellulose barrier layer 100. Layer 101*a* comprises a polymer containing an immobilised enzyme such as urease. Within this layer 101*a*, the urea of the sample is catalytically hydrolysed to ammonium bicarbonate by urease. The NH_4 diffuses into the next layer 102*a* which is a polyvinyl chloride containing an antibiotic such as nonactin as the neutral ion carrier. The NH_4+ is at the interface between layers 101*a* and 102*a*. The next layer 103*a* is a gel containing the electrode couple $Fe(CN)_6^{3-}/Fe/CN)_6^{4-}$ introduced as ammonium salts. The carbon electrode 105*a* lies below layer 103*a*. Electrode 105*a* in contact with layer 103_a serves as the inner reference electrode for the NH_4+ sensor 14*d*. The interfacial potential at the layers 102*a*/103*a* is fixed by the ammonium ferrocyanide salt concentration, and only the interfacial potential of layers 101*a*/102*a* will vary with sample urea concentration.

Electrode 105*b* serves to subtract interferences by measuring the differential of the potential. Layers 101*b*, 102*b*, and 103*b*, respectively, are similar to layers 101*a*, 102*a*, and 103*a*, except that layer 101*b* does not contain urease as its counterpart layer 101*a*.

Layers 104*a* and 104*b* of the sensor are impregnated with a known or predetermined amount of NH_4+ to internally calibrate the sensor sensitivity and compensate for drifts. These layers, similar to the calibration layers in sensor 14*c*, contain high and low levels of the measured species (NH_4+) or alternately the analyte itself (urea).

These predetermined impregnated layers in sensors 14*c* and 14*d* which provide self-calibration, not only assure built-in reliability and accuracy, but relax maufacturing tolerances. Thus, sensor fabrication is greatly facilitated by the built-in calibration.

As aforementioned, many more tests will be performed by the other sensors in the chip array, but all the other sensors, despite their different chemistries, will have the same structure as one of these four sensors (14*a*, 14*b*, 14*c*, and 14*d*). The chip arrays of the invention will always include electrochemical sensors of the invention (such as those illustrated as 14*c* and 14*d*) and may also include other sensors (such as those illustrated as 14*a* and 14*b*). The following Table I is a list of intended measureable analytes and their corresponding sensor structures, i.e., whether they resemble sensor construction for sensors 14*a*, 14*b*, 14*c* or 14*d*, respectively. The immobilised reagents for the various analytes under assay are also given.

TABLE I

Analyte	Enzyme /Substrate	Measurement (*)	Type of Sensor	Species Monitored
I. Electrolytes				
Sodium (Na^+)		P	14c	
Potassium (K^+)		P	14c	
Lithium (Li^+)		A	14a	
Calcium (Ca^{2+})		P	14c	
Chloride (Cl^-)		P	14c	
CO_2 (tot.) (HCO_3—)		P	14c	
Ammonia (NH_3)		P	14c	
II. Gases				
Oxygen (pO_2)		P	14a	
Carbon Dioxide (pCO_2)		P	14c	
pH		P	14c	
Hemoglobin	Catalyst /Redox Mediator	A	14a	
III. Substrates				
Glucose	Glucose Oxidase	A	14a	
Urea (BUN)	Urease	P	14d	
Creatinine	Creatininase	P	14d	
Uric Acid	Uricase	A	14a	
Cholesterol	Cholesterol Oxidase (Cholesterol Hydrolase)	A	14a	
Lactic Acid	LDH	A	14a	
Pyruvic	LDH	A	14a	
Inorganic Phosphorus	Alk. Phosphatase	A	14a	O_2, H_2O_2
Total Proteins	—	P	14d	AG^+
Ascorbic Acid	—	A	14a	direct
Bilirubin	—	A	14a	direct
Triglycerides	Lipase	P	14d	pH
Phenylalanine	Decarboxylase	P	14d	pCO_2
Tyrosine	Decarboxylase	P	14d	PCO_2

TABLE I (Continued)

Analyte	Enzyme /Substrate	Measurement (*)	Type of Sensor	Species Monitored
IV. Enzymes				
Lactic Dehydrogenase (DH)	Lactate /Pyruvate	A	14b	NAD /NADH
Lipase	Triglycerides	A	14b	pH
Amylase	–	A	14a	H_2O_2
Choline Esterase	Acetylcholine	P	14d	pH
GOT	MDH	A	14b	NAD /NADH
GPT	LDH	A	14b	NAD /NADH
CPK	Hexokinase G–6–PD	A	14b	NADP /NADPH
Alk. Phos.	(Phenylphosphate and Piyphenyl-Oxidase).	A	14a	H_2O_2
Acid Phos.	"	A	14a	H_2O_2

* P = Potentiometric Electrode Measurement.

A = Ampermetric or Voltmetric Measurement.

Referring to Figure 11, another embodiment of the integrated chip approach to analyte testing is shown. Chip 10 is replaced by a thin film sensor matrix 10*a*, which comprises sensors 14' having just the electrode structures, redox and calibration layers upon a common substrate. The enzyme layers are absent. Instead, the necessary enzymes for each sensor reaction are contained in a reaction cell or chamber 110. The enzymes are supported upon polymer beads 111 or hollow fibers, etc. The chamber 110 may also be constructed to contain a porous polymer for this purpose.

The sample under analysis is introduced into chamber 110 (arrow 112) via conduit 113 and valve 114. The analytes of the sample each respectively react with their specific enzyme, and are then discharged via valve 115 and conduit 116 to sensor matrix 10*a*.

Each sensor 14' of matrix 10*a* will sense a particular analyte-enzyme reaction as before, i.e., some sensors 14' will measure current, some potential, and some kinetic rate differentials.

After the sensors 14' have accomplished their analyses of the sample, the reaction cell 100 and the matrix 10*a* are washed clean. A first wash liquid is introduced (arrow 121) into conduit 117 and enters chamber 110 via valve 118. The wash is allowed to soak through beads 111 and is discharged (arrow 122) from the chamber 110 via valve 119 and conduit 120. A second wash liquid is then introduced to chamber 110 via conduit 113 and valve 114. The second wash is forced through chamber 110 and is continuously flushed through valve 115 and conduit 116 to matrix 10*a*. The second wash will flow past matrix 10*a* cleaning sensors 14', and then discharges (arrow 123) from the matrix 10*a* via conduit 124.

Naturally, the valves 114, 115, 118 and 119, are respectively opened and closed in proper sequence to accomplish the various sample and wash cycles.

After the second wash, the next sample is introduced into the reaction cell, and the same procedure is followed.

Figures 12—14 illustrate a thin film integrated circuit incorporating sensors of the invention. Figure 12 shows an automatic continuous analysing system 130. A first continuous endless web 131 is stored and dispensed (arrow 133) from reel 132. The web 131 travels past tensioning roller 134 toward a pair of pressure rollers 135. The first endless web 131 comprises discrete partial sensors 140 disposed within a common substrate layer 136 deposited on belt 131 as depicted in Figure 13. Each partial sensor 140 is individually comprised of the necessary gel and polymer layers 141 common to the respective sensors 14*a*, 14*b*, 14*c*, 14*d*, of chip 10. The partial

sensors 140 are each sequentially disposed upon the common substrate 136, but rows 151 of various numbers of partial sensors 140 can be disposed transversely across web 131 as illustrated in Figure 14.

A second continuous web 150 (Figure 12) is advanced (arrow 145) about a frame of rollers 146, 147, 148, and 149, as shown. The second web 150 comprises the electrode structures (not shown) for the corresponding partial sensors 140 of belt 131. When the belts 131 and 150 are advanced and married by pressure rollers 135, a series of completed sensors are formed with structures similar to the sensors 14*a*, 14*b*, 14*c*, etc.

Prior to the completion of the full sensor structures by the pressure rollers 135, either web 131 or web 150 passes a sample dispenser 160. The dispenser 160 is preferably placed over the web 131 (solid lines) instead of web 150 (dotted lines). A drop of sample liquid is dispensed to each partial sensor 140, and permeates the various respective layers 141.

When the electrodes of the web 150 merge with the sample impregnated enzyme layered sensor mediums 140, analytes of the sample will already be reacted. The various signals and information will be conveyed through the electrodes to an analyser 170 as both the merged webs 131 and 150 pass therethrough, as illustrated.

At the rear of the analyser 170, the spent web 131 is discarded (arrow 169). The electrode web 150, however, may be passed by a wash or reconditioning station 168, and can be recycled.

The web 131 may contain an identifying code 161 opposite each particular sensor or sensors 140. This code 161 will be read by the analyser 170 to properly record the analysed data.

Referring to Figure 9, a testing circuit for the enzyme sensor 14*b* of Figure 7*b* is illustrated. The auxiliary electrode 80 and the reference electrode 83*a* will form part of a potential stabilising feedback loop 180 for controlling the voltage between these electrodes. The loop 180 comprises an amplifier 181, which receives an input voltage V_{in}. The applied voltage is sensed at the generating electrode 82. Amplifier 181 supplies the current to the generating electrode 82 via the auxiliary or counter electrode 80.

The sensing electrode 84 is voltage biased at amplifier 182 by $V_{in'}$ and the current is monitored by the amplifier.

The voltage V_s sensed from the generating electrode is given as:

$$V_s = V_{in} - V_{Ref}$$

The voltage V_a applied to the sensing electrode 84 is given as:

$$V_s = V_{In} + V_{In'} - V_{Ref.}$$

Referring to Figures 10 and 10*a*, a schematic of the computer configuration for analyser 30 of Figures 3 and 3*a* is illustrated.

The computer is under the control of the central processor (CPU) 205, which derives its instructions from a stored programme in memory 206, which also contains calibration data for adjusting the processed signals, and stores data in working and permanent storage. The processor 205 does all the arithmetic calculations and processing of the sensor signals. The sensor signals are fed from chip 10 into the analyser 30 via connectors 28 (Figure 3). After an initial conditioning of the signals 201, they are multiplexed by multiplexer 200, and then converted to digital form by the analog-to-digital converter 202. When a particular key 35 (Figure 3) of keyboard 32 is depressed, the key calls for a specific analyte analysis or other appropriate programmed sequence via the process coder 203. The appropriate signal from chip 10 is then processed by the CPU. The processed signal may then be displayed by display 33 and/or a hard copy made by the printer 34. All the signals are properly called up, processed and read under the guidance of the process coder 203. Where desired, an optional set of digital-to-analog converters 207*a*—207*z* will provide an analog input for other peripheral devices. Also, a communication interface 209 can be provided for talking to another computer device, such as a master computer at a data centre.

Figure 10*a* depicts the signal conditioning for signals from a typical sensor 14. The signals from sensor 14 are amplified, biased, and calibrated via the differential amplifier 210 and calibrator controls 211. Then the output 201 from the amplifier 210 is fed to one of the inputs (1 to *n*) of multiplexer 200. The multiplexed signals are fed to the analog/digital converter 202, as aforementioned.

The various techniques for constructing the integrated circuit chip are well known to the practitioners of the electrical arts, but a better understanding of the techniques expressed herein may be obtained with reference to: L.I. Maissel and R. Glang; Handbook of Thin Film Technology; McGraw-Hill Book Co.; Copyright 1970.

Claims

1. An electrochemical sensor for use in the analysis of an analyte in a liquid sample (13), the sensor comprising at least a first and second electrode and means (20) for mounting the said electrodes in spaced relationship, the first electrode (95a; 105b) being electrically connected with a first layer (91a; 104b) of permeable material which contains a given concentration of the species to be measured, and the second electrode (95b; 105a) being electrically connected with a second layer (91b; 104a) of permeable material which is accessible

to the species to be measured as derived from the liquid sample characterised in that the second layer (91b or 104a) is either free from the said species or contains a concentration thereof different from that in the first layer.

2. A sensor according to claim 1, characterised in that the first and second electrodes (95a, b; 105a, b) are mounted on a common support (20) of electrically insulating material.

3. A sensor according to claim 1 or 2, characterised in that the second permeable layer (91b; 104a) is spaced between the second electrode (95b; 105a) and a permeable external face (12c) of the sensor on which in use the liquid sample (13) to be analysed is placed.

4. A sensor according to claim 3, characterised in that one or more further layers (90, 92b, 93b, or 102a, 101a, 103a, 100,) of permeable material are interposed between the said second layer (91b; 104a) and the second electrode (95b; 105a), and/or between the said second layer and the said external face (12c), wherein one or more of said further layers (100 or 90) between the second layer and the external face, are selectively permeable by the analyte to separate said analyte from liquid sample (13), and one or more of said further layers (101a) between the second layer and the second electrode contain substances for converting analyte into the species to be measured.

5. Apparatus for simultaneously analysing a multiplicity of analytes in a fluid sample, the apparatus comprising an array of discrete, electrically isolated electrochemical sensors (14) for analysing different analytes, all the sensors being supported on a common substrate (20), and electrical circuitry (24) for obtaining the signal(s) from each sensor, characterised in that at least one of the sensors (14) is as claimed in any of claims 1 to 4.

6. Apparatus according to claim 5, characterised in that the electrical circuitry (24) comprises electrical conductors (25) supported on said substrate (20), said conductors extending from respective sensors (14) to one or more connectors (27) at the periphery of said substrate.

7. Apparatus according to claim 6, characterised in that the electrical conductors (25) have been printed on said substrate (20).

8. Apparatus according to claim 7, characterised in that some of said sensors (14) comprise printed electrodes deposited on said substrate in electrical continuity with respective conductors.

9. Apparatus according to claim 6, 7, or 8, characterised in that the or each connector (27) is adapted to be received in a snap-fit receiver (28) of an analysing means (30) responsive to at least selective ones of said sensors (14).

10. Apparatus according to any of claims 1 to 9, characterised in that at least one of the electrodes (95a, b; 105a, b) is of carbon.

11. Apparatus according to any of claims 1 to 10, characterised in that the substrate (20) is of pressed alumina powder.

12. Apparatus for use in analysing a multiplicity of analytes in a fluid sample (13), comprising a plurality of discrete, electrically isolated sensors in accordance with claim 1 adapted to receive reaction products, said sensors (14) being supported on a common substrate, each of said sensors being specific to a particular reaction product, characterised by a reaction cell (110) for selectively reacting particular constituents in a liqud sample to form said reaction products with each of said constituents.

13. Apparatus according to any of claims 5 to 9, which includes in addition to the sensors (14c, 14d) in accordance with claim 1, one or more further electrochemical sensors (14a, 14b) characterised in that the said further sensors include a sensor (14b) for measuring enzyme analytes which comprises a generating electrode (82), a monitoring electrode (84) and a reaction medium (81) disposed therebetween and containing the enzyme substrate and a co-enzyme for supporting a reaction of the enzyme being analysed, the generating electrode being such that it can be supplied with constant current to provide electrochemically in said medium a controlled rate of formation of a reactant of said enzyme reaction, and the monitoring electrode being such that during said enzyme reaction as the said reactant is consumed, it measures the net generation rate of the reactant in the medium.

14. Apparatus according to any of claims 5 to 9 or 12, characterised in that each said sensors have been formed by pressing into contact a first web (131) comprising a plurality of partial sensors (140), and a second web (150) comprising a corresponding plurality of electrode structures, to form plurality of discrete electrically isolated sensors comprising sensors in accordance with claim 1.

Revendications

1. Capteur électrochimique destiné à être utilisé pour l'analyse d'un composant dans un échantillon de liquide (13), le capteur comportant au moins une première et une seconde électrodes et des moyens (20) pour monter lesdites électrodes à distance l'une de l'autre, la première électrode (95*a*; 105*b*) étant reliée électriquement à une première couche (91*a*; 104*b*) en matériau perméable qui contient une concentration donnée du corps devant être mesuré, et la seconde électrode (95*b*; 105*a*) étant reliée électriquement à une seconde couche (91*b*; 104*a*) de matériau perméable et auxquelles peuvent avoir accès le corps à mesurer tel qu'il est tiré de l'échantillon de liquide, caractérisé en ce que la seconde couche (91*b* ou 104*a*) est soit dépourvue dudit corps, soit contient une concentration de ce dernier différente de celle présente dans la première couche.

2. Capteur selon la revendication 1, caractérisé en ce que les première et seconde électrodes (95*a*, *b*; 105*a*, *b*) sont montée sur un support commun (20) en un matériau électriquement isolant.

3. Capteur selon l'une des revendications 1 ou 2, caractérisé en ce que la seconde couche perméable (91*b*; 104*a*) est montée à distance entre la seconde électrode (95*b*; 105*a*) et une face extérieure perméable (12*c*) du capteur sur lequel, en cours d'utilisation, l'échantillon de liquide (13) devant être analysé est disposé.

4. Capteur selon la revendication 3, caractérisé en ce qu'une ou plusieurs couches (90, 92*b*, 93*b* ou 102*a*, 101*a*, 103*a*, 100) de matériau perméable sont interposées entre la seconde couche (91*b*; 104*a*) et la seconde électrode (95*b*; 105*a*) et/ou entre la seconde couche et ladite face extérieure (12*c*), une ou plusieurs desdites autres couches (100 ou 90), situées entre la seconde couche et la face extérieure, pouvant être traversées de façon sélective par le composant de manière à permettre une séparation dudit composant de l'échantillon de liquide (13), et une ou plusieurs desdites autres couches (101*a*), situées entre la seconde couche et la seconde électrode, contenant des substances pour transformer le composant en le corps devant être mesuré.

5. Appareil pour réaliser l'analyse simultanée d'une multiplicité de composants dans un échantillon de fluides, l'appareil comportant un réseau de capteurs électrochimiques discrets (14) isolés électriquement pour analyser différents composants, tous les capteurs étant portés par un substrat commun (20), et un circuit électrique (24) permettant d'obtenir le ou les signaux de la part de chaque capteur, caractérisé en ce qu'au moins l'un des capteurs (14) est tel que revendiqué dans l'une quelconque des revendications 1 à 4.

6. Appareil selon la revendication 5, caractérisé en ce que le circuit électrique (24) comporte des conducteurs électriques (25) portés par ledit substrat (20), lesdits conducteurs s'etendant depuis des capteurs respectifs (14) vers un ou plusieurs connecteurs (27) situés sur le pourtour dudit substrat.

7. Appareil selon la revendication 6, caractérisé en ce que les conducteurs électriques (25) ont été imprimés sur ledit substrat (20).

8. Appareil selon la revendication 7, caractérisé en ce que certains des capteurs (14) comportent des électrodes imprimées déposées sur ledit substrate et établissant une continuité électrique avec les conducteurs respectifs.

9. Appareil selon la revendication 6, 7 ou 8, caractérisé en ce que le ou chaque connecteur (27) peut être logé dans un logement (28) à encliquetage brusque d'un dispositif analyseur (30) en réponse à au moins certains capteurs sélectifs desdits capteurs (14).

10. Appareil selon l'une quelconque des revendications 1 à 9, caractérisé en ce qu'au moins l'une des électrodes (95*a*, *b*; 105*a*, *b*) est en carbone.

11. Appareil selon l'une quelconque des revendications 1 à 10, caractérisé en ce que le substrat (20) est constitué par de la poudre d'alumine comprimé.

12. Appareil destiné à être utilisé pour l'analyse d'une multiplicité de composants dans un échantillon de fluide (13), comportant plusieurs capteurs discrets isolés électriquement conformément à la revendication 1, aptes à recevoir des produits de réaction, lesdits capteurs (14') étant portés par un substrat commun et chacun d'eux étant spécifique à un produit de réaction particulier, caractérisé par une cellule réactionnelle (110) permettant de faire réagir sélectivement des constituants particuliers dans un échantillon de liquide pour former lesdits produits de réaction avec chacun desdits constituants.

13. Appareil selon l'une quelconque des revendications 5 à 9 incluant, en plus des capteurs (14*c*, 14*d*) conformément à la revendication 1, un ou plusieurs autres capteurs électrochimiques (14*a*, 14*b*), caractérisé en ce que les autres capteurs comprennent un capteur (14*b*) pour la mesure de composants enzymatiques qui comporte une électrode génératrice (82), une électrode de contrôle (84) et un milieu réactionnel (81) interposé entre ces électrodes et contenant le substrat enzymatiques et un co-enzyme, pour favoriser une réaction de l'enzyme analysé, l'électrode génératrice étant telle qu'elle peut être alimentée par un courant constant de manière à fournir par voie électrochimique, dans ledit milieu, une vitesse commandée de formation d'un corps en réaction de ladite réaction enzymatique, et l'électrode de contrôle étant telle que pendant ladite réaction enzymatique, lorsque le corps en réaction est consommé, elle mesure la vitesse nette de production du corps en réaction.

14. Appareil selon l'une quelconque des revendications 5 à 9 ou 12, caractérisé en ce que chacun desdits capteurs a été formé par compression en contact avec une première bande (131) comportant une pluralité de capteurs partiels (140), et une seconde bande (150) comportant une pluralité correspondante de structures d'électrodes pour former plusieurs capteurs discrets isolés électriquement, comprenant des capteurs conformes à la revendication 1.

Patentansprüche

1. Elektrochemischer Sensor zur Verwendung bei der Analyse eines zu analysierenden Bestandteils in einer Flüssigkeitsprobe (13), bestehend aus mindestens einer ersten und einer zweiten Elektrode sowie Mitteln (20) zum Montieren der Elektroden im Abstand voneinander, wobei die erste Elektrode (95a; 105b) elektrisch mit einer ersten Schicht (91a; 104b) aus

durchlässigem Material, das eine gegebene Konzentration des zu messenden Bestandteils enthält, und die zweite Elektrode (95b; 105a) elektrisch mit einer zweiten Schicht (91b; 104a) aus durchlässigem Material, das für den zu messenden Bestandteil aus der Flüssigkeitsprobe zugänglich ist, verbunden ist, dadurch gekennzeichnet, daß die zweite Schicht (91b oder 104a) entweder frei von dem Bestandteil ist oder davon eine Konzentration enthält, die sich von der Konzentration in der ersten Schicht unterscheidet.

2. Sensor gemäß Anspruch 1, dadurch gekennzeichnet, daß die erste und zweite Elektrode (95a, b; 105a, b) auf einem gemeinsamen Träger (20) aus elektrisch isolierendem Material montiert sind.

3. Sensor gemäß Anspruch 1 oder 2, dadurch gekennzeichnet, daß die zweite durchlässige Schicht (91b; 104a) zwischen der zweiten Elektrode (95b; 105a) und einer durchlässigen äußeren Fläche (12c) des Sensors, auf die während des Betriebes die zu analysierende Flüssigkeitsprobe (13) aufgebracht wird, jeweils im Abstand angeordnet ist.

4. Sensor gemäß Anspruch 3, dadurch gekennzeichnet, daß zwischen der zweiten Schicht (91b; 104a) und der zweiten Elektrode (95b; 105a) und bzw. oder zwischen der zweiten Schicht und der Außenfläche (12c) eine oder mehrere weitere Schichten (90, 92b, 93b, oder 102a, 101a, 103a, 100) aus durchlässigem Material angeordnet sind, wobei eine oder mehrere der weiteren Schichten (100 oder 90) zwischen der zweiten Schicht und der Außenfläche selektive für den nachzuweisenden oder zu bestimmenden Bestandteil durchlässig sind, um den nachzuweisenden oder zu bestimmenden Bestandteil von der Flüssigkeitsprobe (13) abzutrennen, und eine oder mehrere der weiteren Schichten (101a) zwischen der zweiten Schicht und der zweiten Elektrode Substanzen zum Überführen des nachzuweisenden oder zu bestimmenden Bestandteiles in einen zu messenden Stoff enthalten.

5. Vorrichtung zum gleichzeitigen Nachweis bzw. zur gleichzeitigen Bestimmung einer Mehrzahl von Bestandteilen in einer Flüssigkeitsprobe, bestehend aus einer Reihe diskreter, elektrisch isolierter elektrochemischer Sensoren (14) zum Nachweis oder zur Bestimmung unterschiedlicher nachzuweisender oder zu bestimmender Bestandteile, wobei sämtliche Sensoren von einem gemeinsamen Trägersubstrat (20) gehaltert sind, sowie aus einem elektrischen Schaltungsaufbau (24) zum Erzielen eines Signals bzw. von Signalen von jedem Sensor, dadurch gekennzeichnet, daß mindestens einer der Sensoren (14) ein solcher gemäß einem der Ansprüche 1 bis 4 ist.

6. Vorrichtung gemäß Anspruch 5, dadurch gekennzeichnet, daß der elektrische Schaltungsaufbau (24) elektrische Leiter (25) umfaßt, die auf dem Trägersubstrat (20) gehaltert sind und die sich von den entsprechenden Sensoren (14) zu einem oder mehreren Anschlüssen (27) im Außenbereich des Substrates erstrecken.

7. Vorrichtung gemäß Anspruch 6, dadurch gekennzeichnet, daß die elektrischen Leiter (25) auf das Trägersubstrat (20) aufgedruckt sind.

8. Vorrichtung gemäß Anspruch 7, dadurch gekennzeichnet, daß einige der Sensoren (14) gedruckte Elektroden enthalten, die auf das Trägersubstrat in elektrischer Kontinuität mit den entsprechenden Leitern abgeschieden sind.

9. Vorrichtung gemäß Anspruch 6, 7 oder 8, dadurch gekennzeichnet, daß jeder Anschluß (27) derart eingerichtet ist, daß er in einen Schnappstecker (28) einer Analysiervorrichtung (39) paßt, die auf mindestens ausgewählte Sensoren (14) anspricht.

10. Vorrichtung gemäß einem der Ansprüche 1 bis 9, dadurch gekennzeichnet, daß mindestens eine der Elektroden (95a, b; 105a, b) aus Kohlenstoff besteht.

11. Vorrichtung gemäß einem der Ansprüche 1 bis 10, dadurch gekennzeichnet, daß das Halterungssubstrat (20) aus gepreßtem Aluminiumoxid-Pulver besteht.

12. Vorrichtung zum Nachweis oder zur Bestimmung einer Vielzahl von nachzuweisenden oder zu bestimmenden Bestandteilen in einer Flüssigkeitsprobe (13), bestehend aus einer Vielzahl diskreter, elektrisch isolierter Sensoren gemäß Anspruch 1, die Reaktionsprodukte aufnehmen und die (14') auf einem gemeinsamen Trägersubstrat gehaltert sind, wobei jeder Sensor spezifisch hinsichtlich eines bestimmten Reaktionsproduktes ist, gekennzeichnet durch eine Reaktionszelle (110) zur selektiven Umsetzung bestimmter Bestandteile in einer Flüssigkeitsprobe unter Ausbildung der Reaktionsprodukte mit jedem dieser Bestandteile.

13. Vorrichtung gemäß einem der Ansprüche 5 bis 9, die zusätzlich zu den Sensoren (14c, 14d) gemäß Anspruch 1 einen oder mehrere weitere elektrochemische Sensoren (14a, 14b) enthält, dadurch gekennzeichnet, daß die weiteren Sensoren einen Sensor (14b) zum Messen von Enzymbestandteilen enthalten, der aus einer erzeugenden Elektrode (82), einer Überwachungselektrode (84) sowie einem dazwischen angeordneten und das Enzymsubstrat und ein Koenzym enthaltenden Reaktionsmedium (81) zur Unterhaltung einer Reaktion des zu messenden Enzyms besteht, wobei die erzeugende Elektrode derart ausgebildet ist, daß sie mit konstantem Strom versorgt werden kann, um in dem Medium elektrochemisch eine gesteuerte Bildungsgeschwindigkeit eines Reaktionsteilnehmers der Enzymreaktion zu bewirken, und die Überwachungselektrode derart ausgebildet ist, daß sie während der Enzymreaktion in dem Maße, wie der Reaktionsteilnehmer verbraucht wird, die Nettoerzeugungsgeschwindigkeit des Reaktionsteilnehmers in dem Medium, mißt.

14. Vorrichtung gemäß einem der Ansprüche

5 bis 9 oder 12, dadurch gekennzeichnet, daß jeder der Sensoren dadurch gebildet worden ist, daß man ein erstes Gewebe (131) aus einer Anzahl von Teilsensoren (140) und ein zweites Gewebe (150) aus einer entsprechenden Anzahl von Elektrodenstrukturen zu einer Anzahl diskreter, elektrisch isolierter Sensoren gemäß Anspruch 1 zusammenpreßt.

FIG. 1

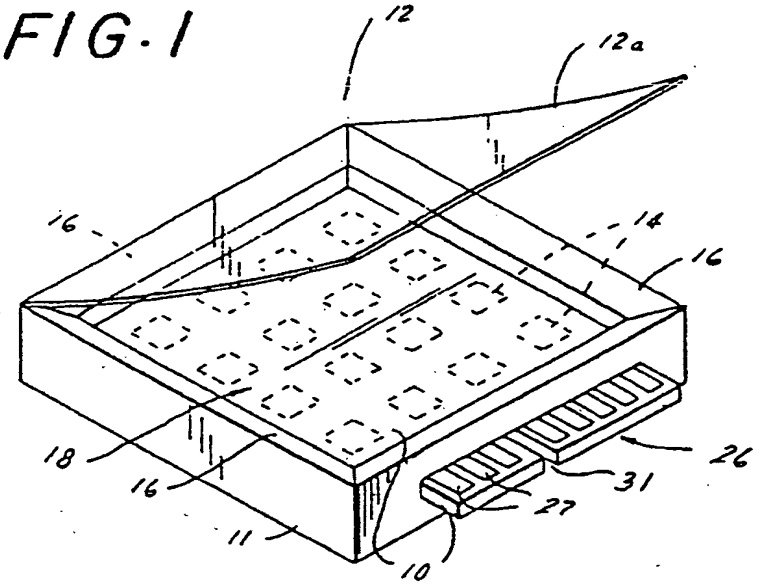
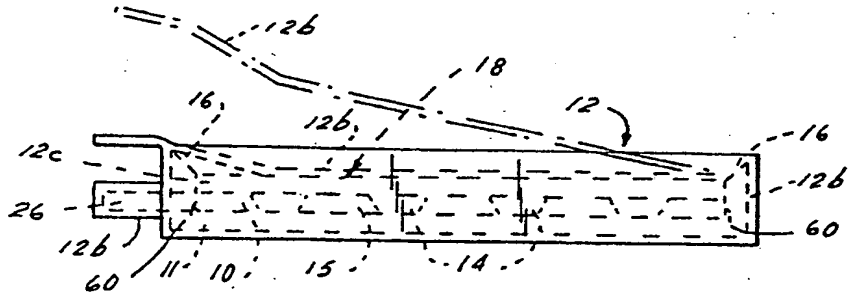


FIG. 1a



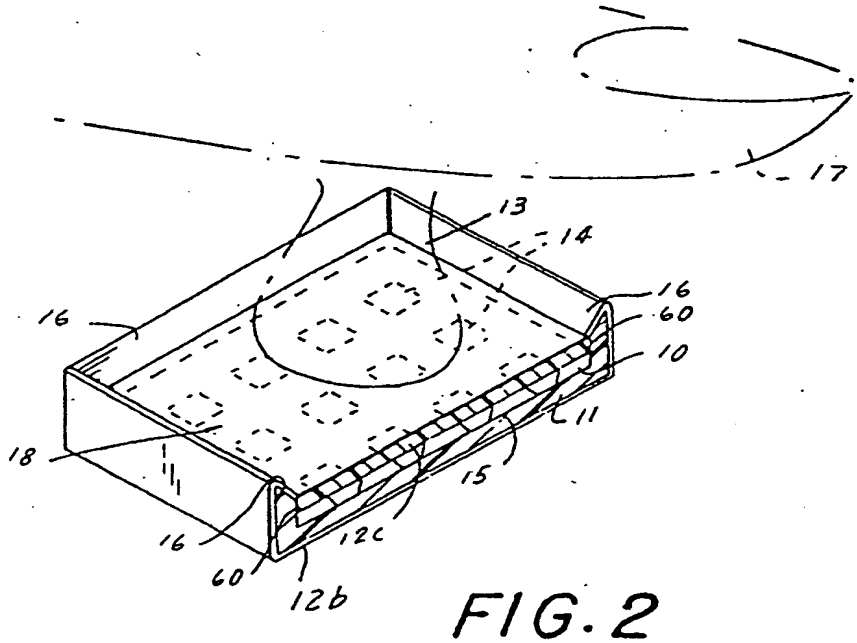


FIG. 2

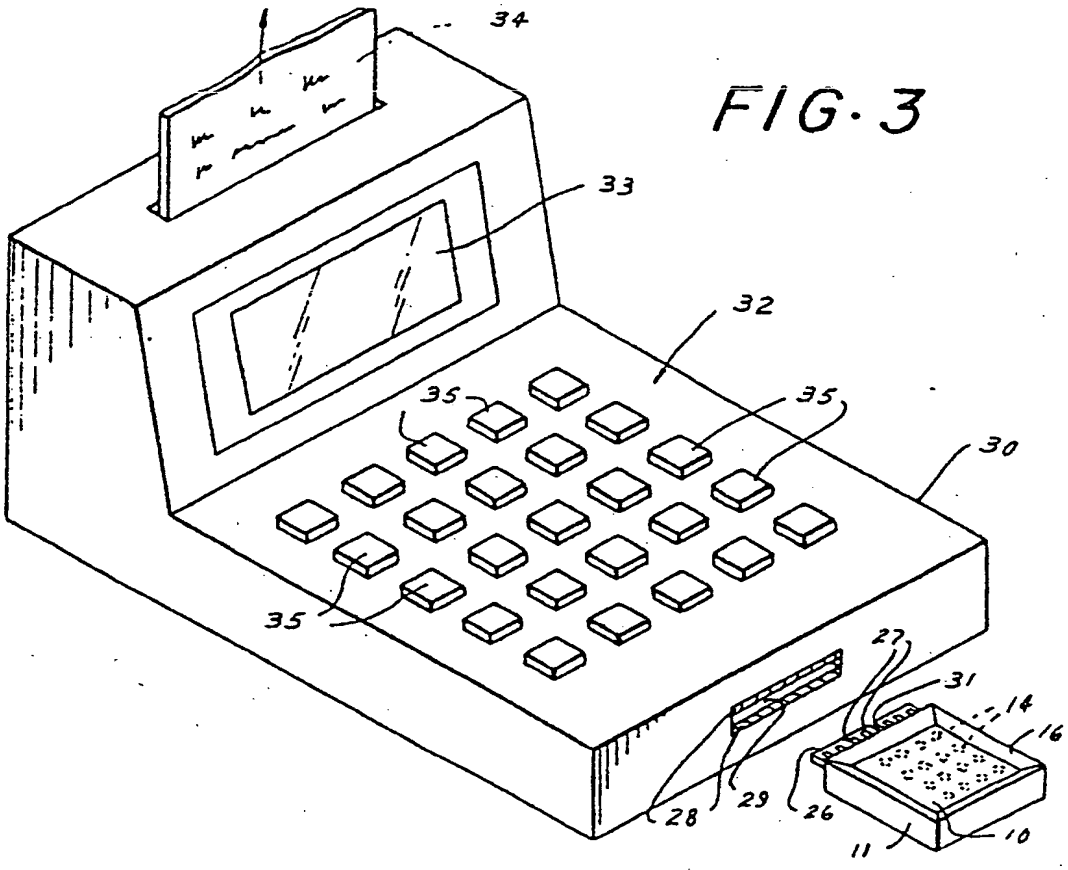

34
33
32
35
35
35
30
27
31
14
16
28
29
26
11
10

FIG. 3

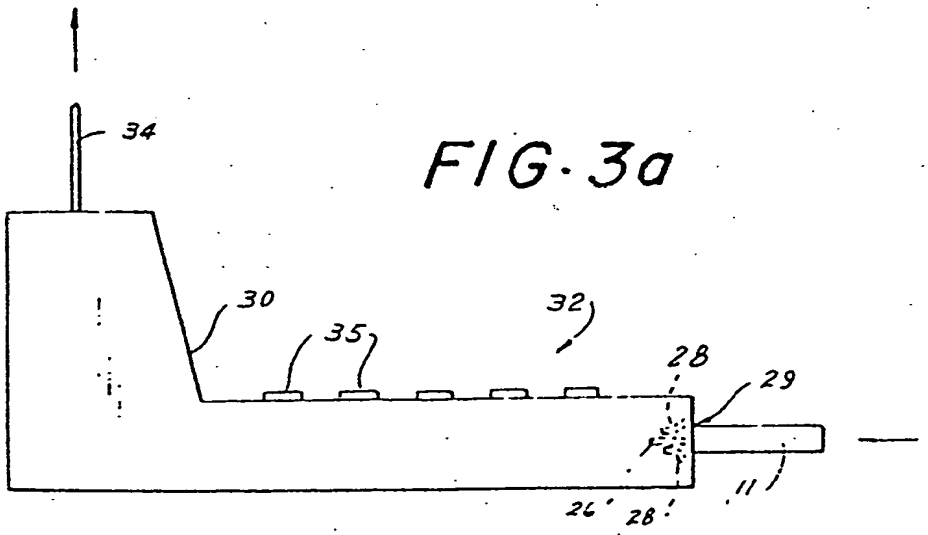
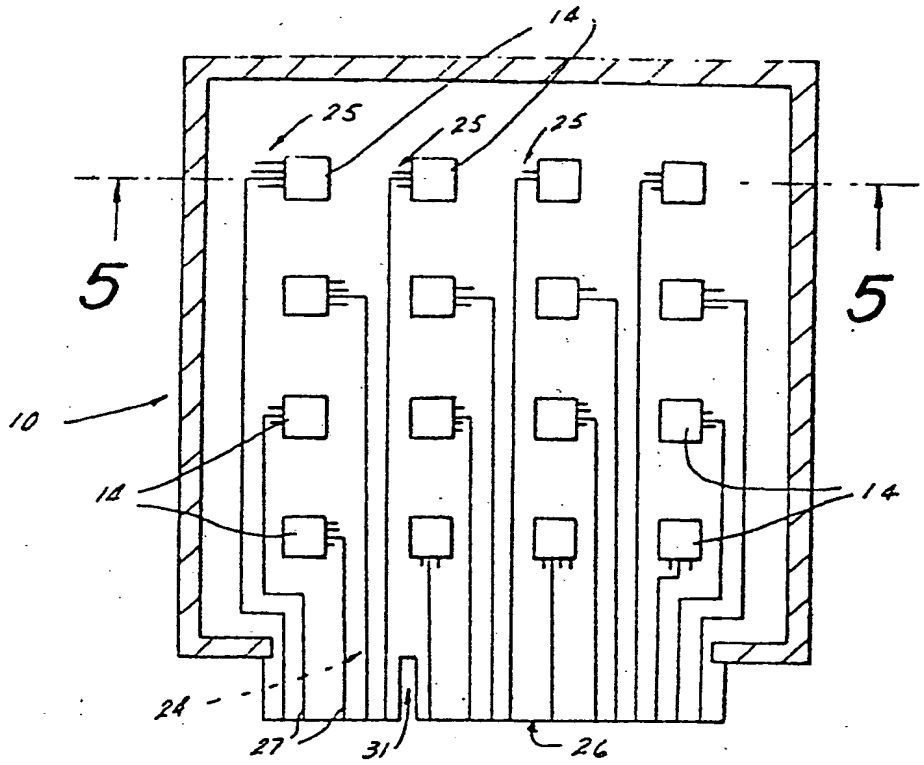

34
30
35
32
28
29
11
26'
28'

FIG. 3a

FIG. 4



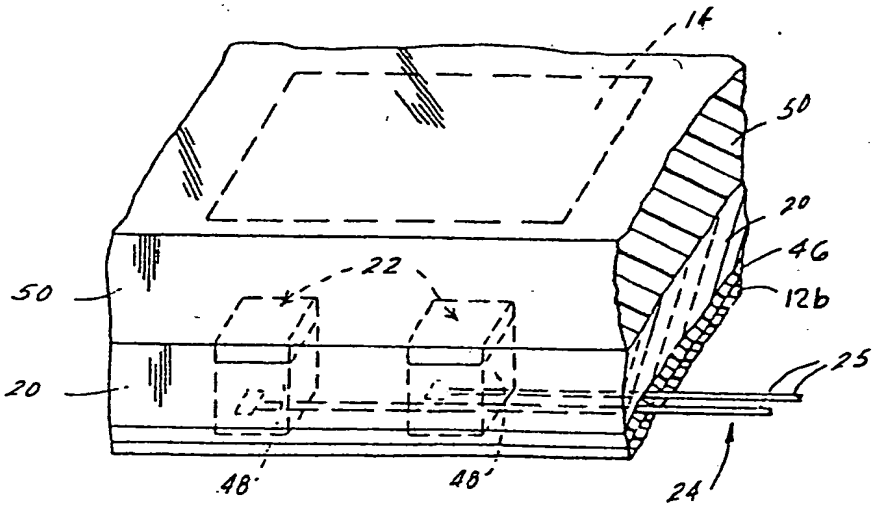


FIG. 8a

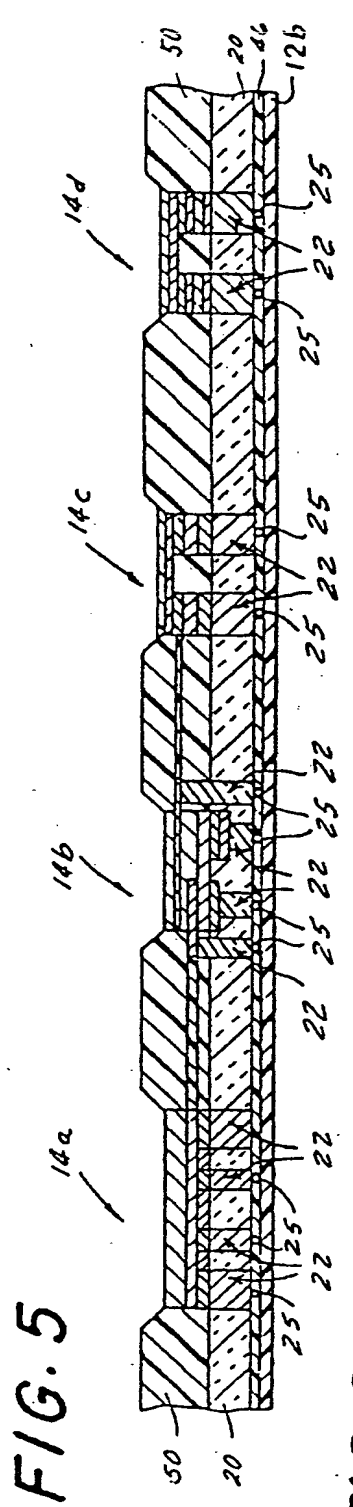


FIG. 5

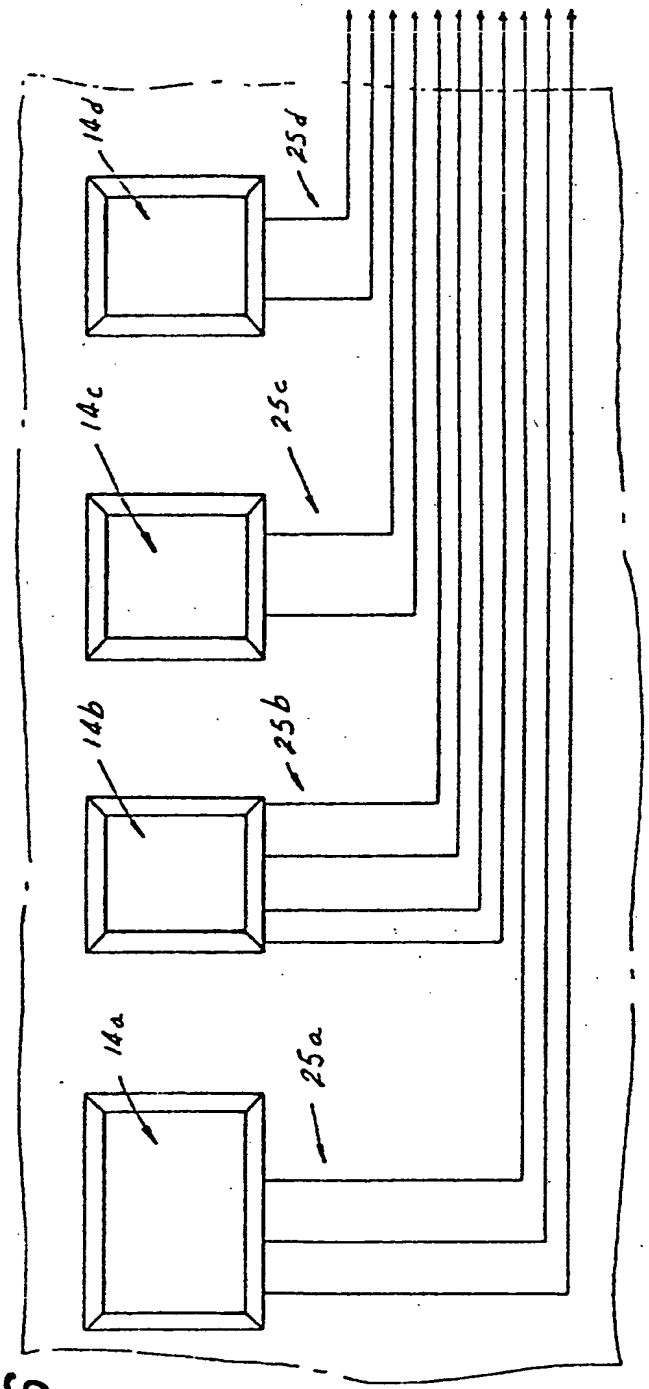
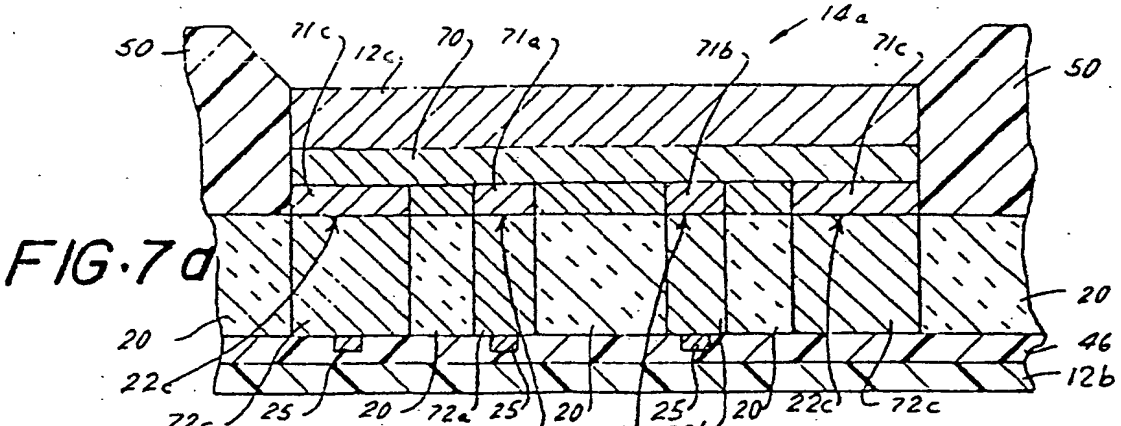
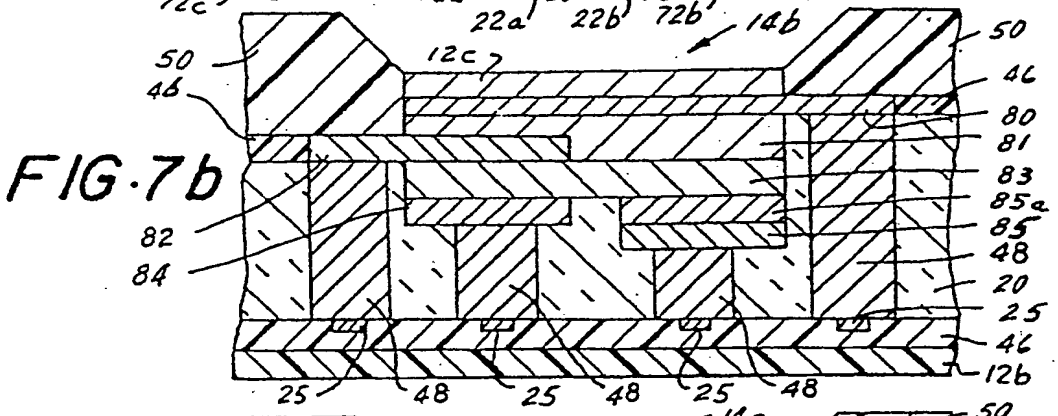


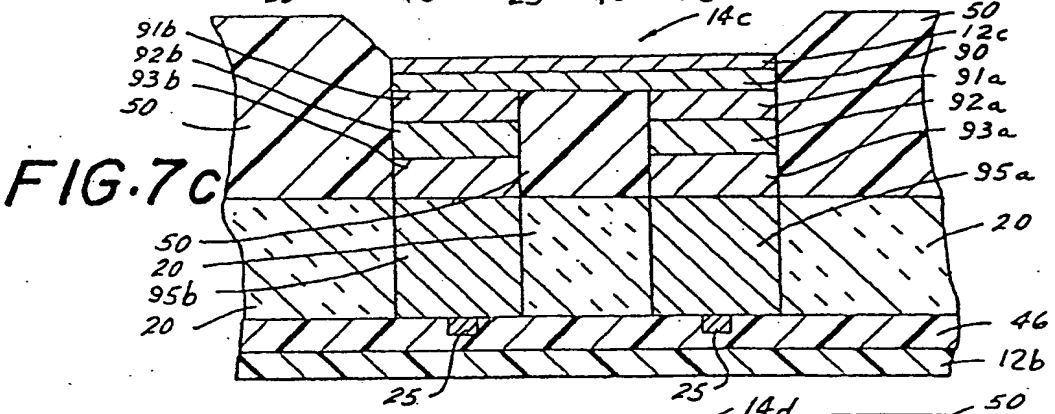
FIG. 6



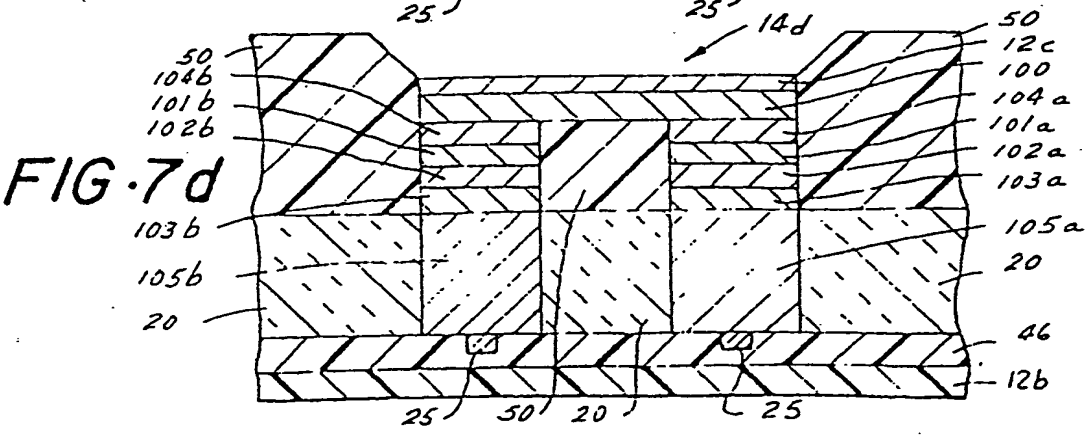
FIG·7a



FIG·7b



FIG·7c



FIG·7d

FIG. 8

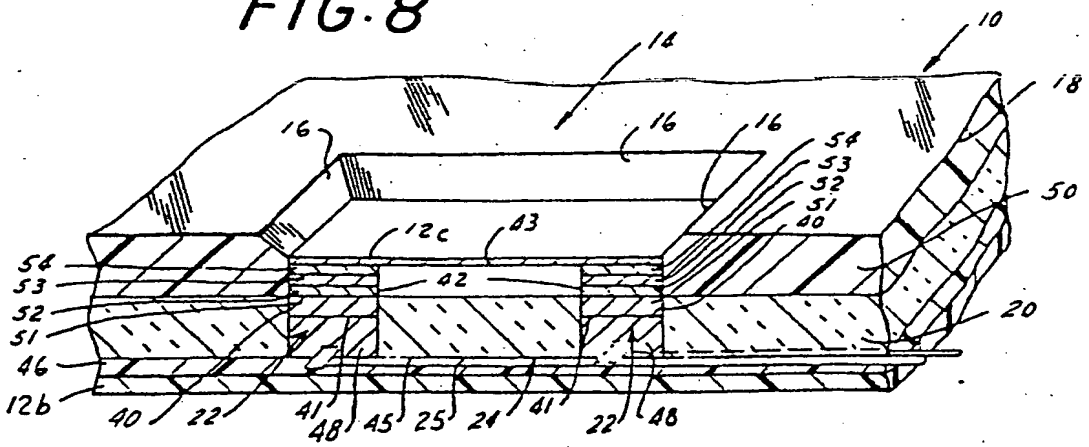


FIG. 9

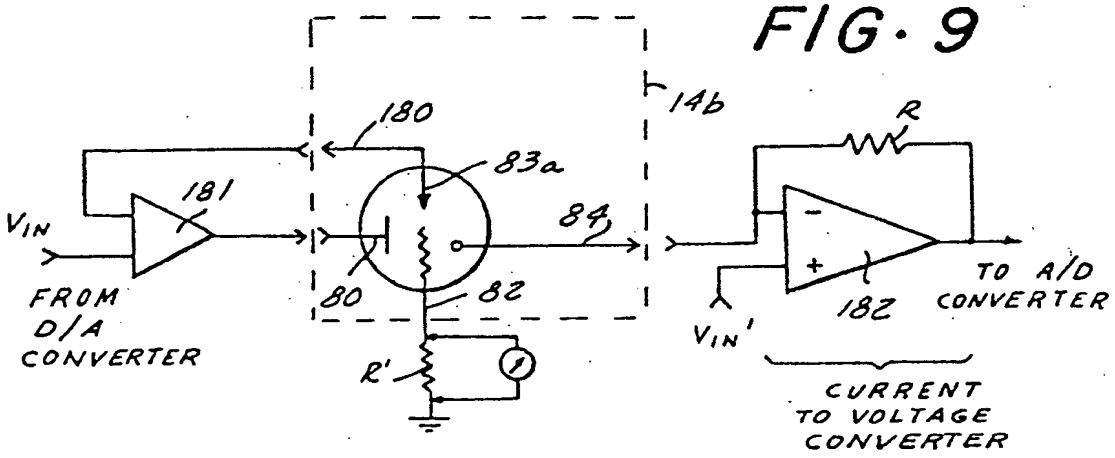
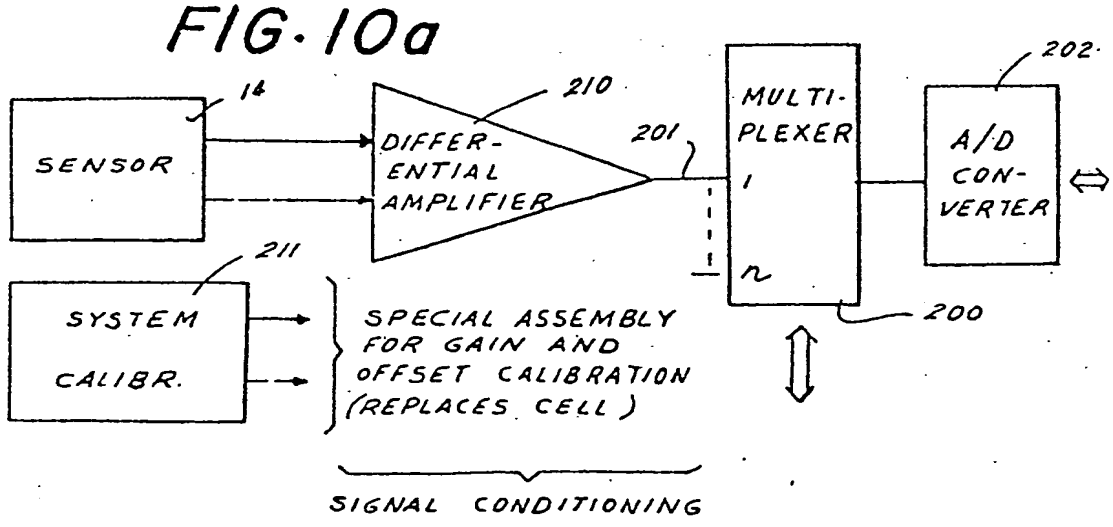


FIG. 10a



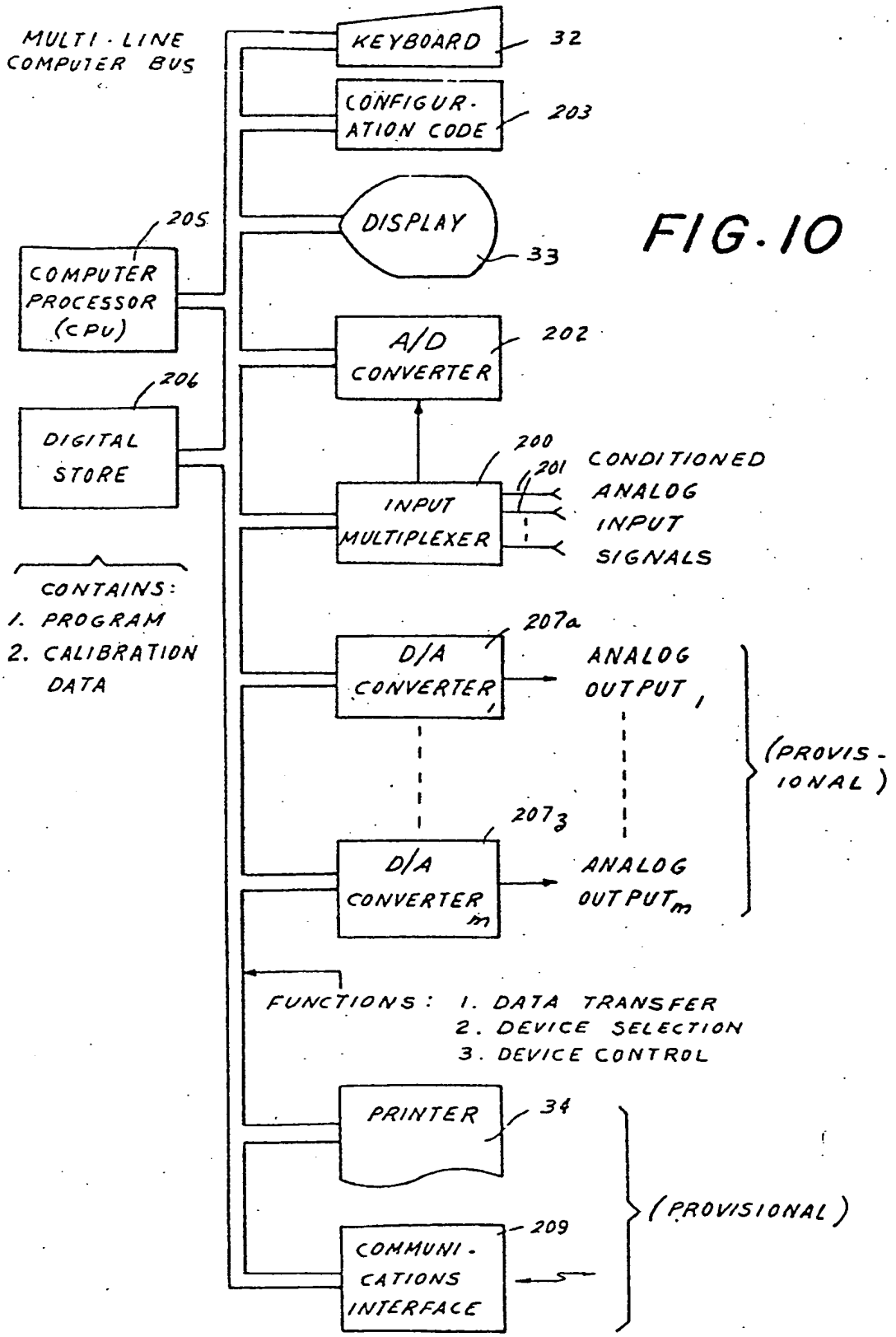
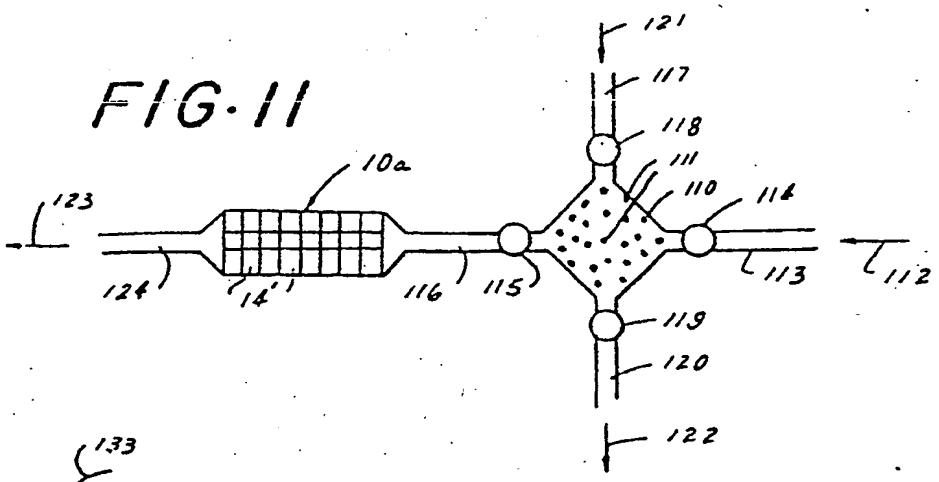
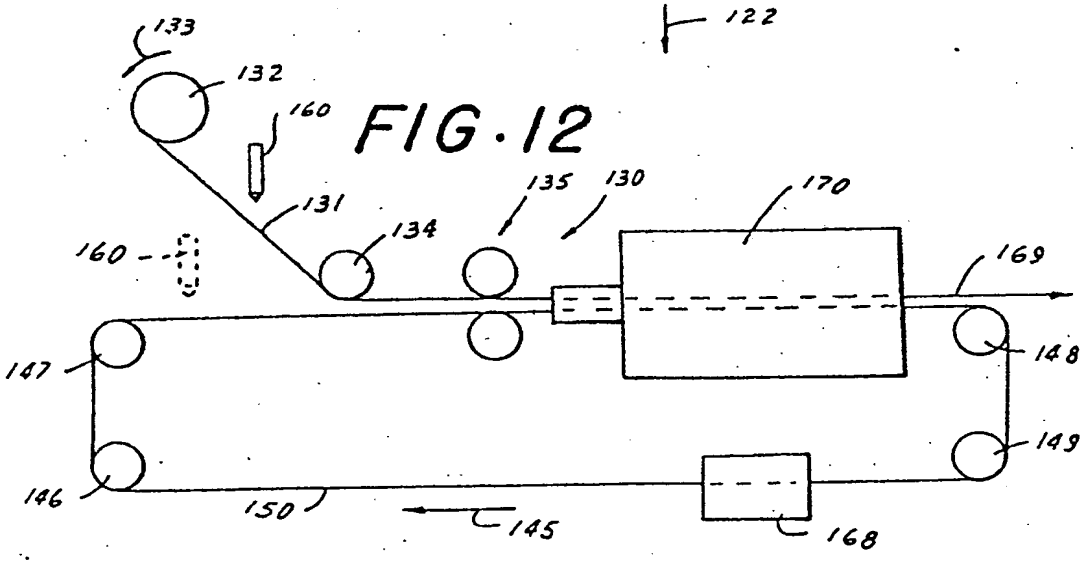

MULTI-LINE COMPUTER BUS
KEYBOARD
32
CONFIGUR-ATION CODE
203
DISPLAY
33
205
COMPUTER PROCESSOR (CPU)
A/D CONVERTER
202
206
DIGITAL STORE
200
201
CONDITIONED ANALOG INPUT SIGNALS
INPUT MULTIPLEXER
CONTAINS:
1. PROGRAM
2. CALIBRATION DATA
207a
D/A CONVERTER 1
ANALOG OUTPUT 1
(PROVIS-IONAL)
207m
D/A CONVERTER m
ANALOG OUTPUT m
FUNCTIONS: 1. DATA TRANSFER
2. DEVICE SELECTION
3. DEVICE CONTROL
PRINTER
34
209
COMMUNI-CATIONS INTERFACE
(PROVISIONAL)

FIG. 10

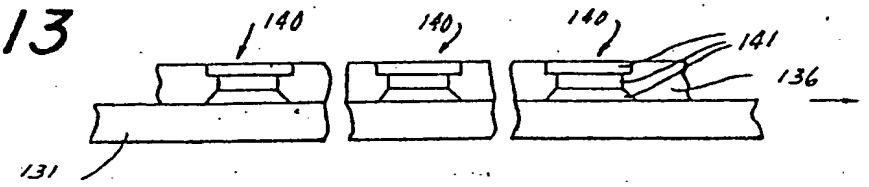
FIG·11



FIG·12



FIG·13



FIG·14